Available online on www.ijpqa.com
International Journal of Pharmaceutical Quality Assurance 2019; 10(2); 311-314
doi: 10.25258/ijpqa.10.2.16

ISSN 0975 9506

Research Article

Isolation and Identification of *Bacteroides fragilis* in Baghdad

Luma Yousif Mehdi*, Mayada Noori Iqbal, Zeina Anwar Jaffar

Health and Medical Technical College/ Baghdad,Middle Technical University/ Iraq

Received: 1st Mar, 19; Revised: 12th Apr, 19, Accepted: 26th Apr, 19; Available Online: 25th Jun, 2019

ABSTRACT

Bacteroides fragilis are anaerobic bacteria that cause diversified infections such as diarrhea and colorectal cancer, intra-abdominal infection, postoperative wound infection, and the most antibiotic-resistant among the anaerobic bacteria. This study for determination of the prevalence of *B. fragilis* from patients suffering from diarrhea, Colorectal cancer, Abdominal surgical abscesses and Vaginal inflammation and to determine the resistance of isolated against antibiotics. Samples of (145) patients with different casese were taken from Baghdad hospitals, by using culture, Api 20A kit and PCR were used to detect and confirm isolation and identification of *B. Fragilis*, disk diffusion was performed for antibiotic resistance. The prevalence rates of infection cases increased among children suffering from diarrhea were (11.7%), and colorectal cancer among elderly people were (11%), abdominal surgical abscesses were (9.7%) respectively, and resistance to most antibiotics. This study revealed that the *B. Fragilis* are important pathogens that frequently cause various infections, the antimicrobial resistance has accretion. The future advances research should explain the epidemiology of enterotoxigenic and also participate to the prevention of outbreaks human diseases.

Keyword: *B. fragilis*, Api20A system, PCR, sensitivity to antibiotics.

INTRODUCTION

Bacteroides fragilis is Gram-negative bacillus, an anaerobic and a normal flora community of the human gastrointestinal member of the tract and vagina and illustrate a common cause of endogenous infections in humans that is repeatedly associated with infections such as intraabdominal, diabetic foot, obstetric-gynecologic tract and surgical site infections, as well as toxin-dependent diarrhea in adults and children[1,2]. Enterotoxigenic *B. fragilis* (ETBF) induced diarrhea in children has been reported by researchers in several parts of the world[3,4] and associated with acute diarrheal disease, inflammatory bowel disease, and colorectal cancer (CRC)and established as a cause of diarrheal disease in all age groups globally now, with most reports focusing on young children and could be a cause for vaginitis. The *bft* gene is associated with colorectal neoplasia, especially in late-stage CRC and may be a risk agent for developing CRC. The option of antibiotics for curing are limited because of *B. Fragilis* are among the most resistant anaerobic to antimicrobial agents[5,6,7], and because reduction national data about the prevalence species of *B. fragilis* and their susceptibility to antibiotics in our city, so that the purpose of this work was to determine the prevalence of *B. Fragilis* in various samples and their antibiotic susceptibility.

METHODS

One hundred fourty five (145) patient samples from different sources were collected from Baghdad hospitals (Child protection teaching hospital, Baghdad teaching hospital, Private Nursing Home Hospital, Children teaching hospital). Sample were taken during the period of first of March 2018 till the end of September 2018.

Isolation of *B. Fragilis*: all samples were taken by sterile disposable cotton swabs, then inserted into Brain heart broth (Oxoid, UK) as transport media and take to the laboratory, each swab was spread on the surface of Columbia agar supplemented with { 5% blood, hemin 0.005 g/L and vitamin K 0.01 g/L, (Sigma Aldrich, UK)} ,and mycostatin (240 U/L), vancomycin (0.004 mg/L, Amikacin (0.01 g/L)(Oxoid, England), and Kanamycin-Vancomycin – Laked Blood (KVLB, Basal Medium is Brucella agar (Fluka) incubated anaerobically by Gas pack at 37C° for 24 to 48hrs. by anaerobic jar (Oxoid Anaerobic Jar with Anaerogen (AN0025,OXOID,UK) gas back Kit. The pure bacterial suspensions were used for API 20A biochemical to confirm identification tests[8] (API 20A KIT, bioMerieux, Inc. USA) (Table:3). Colonial and microscopic morphology, Gram stain, and the enterotoxin gene (*bft*) was detected by PCR to confirm and complete the identification.

Antibiotic dick diffusion methods was performed with (Oxoid disks, UK). Antimicrobial susceptibility testing to six antimicrobials (Ampicillin, Vancomycin, Erythromycin, Metronidazole, Chloramphenicol, Rifampin) were carried out for (22) isolated by the disk diffusion method on Muellere Hinton agar with 5%

*Author for Correspondence: yousifnawar@yahoo.com

Table 1: Distribution of *B. Fragilis* Infections According to patients cases and difference between the methods for identification and conformation isolates .

Patients cases	Age groups /years	Patients No.	Samples presumptively Positive Isolations No.(%) by culture and Api20A	Confirmation isolated by PCR
	5 and less	41	7(13.7%)	5(9.8%)
Diarrhea	6-10	7	1(1.9%)	1(1.9%)
	11-15	3	0.00	0.00
	Total No.	51	8(15.6%)	6(11.7%)
	60-70	22	3(6.7%)	2(4.4%)
Stool specimens from	71-80	23	4(8.9%)	3(6.6%)
Colorectal cancer	Total No.	45	7(15.6)	5(11%)
Abdominal surgical	25-35	16	3(9.7%)	2(6.5%)
Abscesses	35-45	15	1(3.2%)	1(3.2%)
	Total No.	31	4(12.9%)	3(9.7%)
	25-35	10	1(5,6%)	000
Vaginal inflammation	35-45	8	1(5,6%)	1(5,6%)
	Total No.	18	2(11%)	000

Table 2: Api20A identification results of 22 isolates.

Test	Results
Indole (IND)	−
Urease (URE)	−
Glucose (GLU)	+
Manitol (MAN)	−
Lactose (LAC)	+
Saccharose (SAC)	+
Maltose (MAL)	+
Salicin (SAL)	−
Xylose (XYL)	+
Arabinose (ARA)	−
Hydrolysis(protease)(gelatin)GEL	−
Hydrolysis(β-glucosidase) (esculin)ESC	+
Glycerol (GLY)	−
Cellobiose (CEL)	−
Mannose (MNE)	+
Melezitose (MLZ)	−
Raffinose (RAF)	+
Sorbitol (SOR)	−
Rhamnose (RHA)	−
Trehalose (TRE)	−
Catalase (CAT)	+
Spores (SPOR)	−
GRAM reaction	−
COCC morphology	−

_ negative\ +positive

sheep blood (HiMedia, India). The plates were incubated at 37°C for 24 hrs. anaerobically. The inhibition zone was measured for each antibiotic were determined according to BSAC methods for antimicrobial susceptibility testing[9].

DNA extraction

Bacterial genomic DNA was extracted from BHI broth samples by employment of Genomic DNA Purification kit (Promega, USA).

The enterotixin gene was amplified by PCR amplification **:** the primers used were (forward, 5 ᾽-GAG CCG AAG ACG GTG TAT GTG ATT TGT-) and (reverse, 5'-TGC TCA GCG CCC AGT ATA TGA CCT AGT-) primer pairs (Alpha DNA, Canada) which were described previously[10], expected products of amplification was 400 bp.

PCR amplifications were carried out in total reaction volumes of 50µl containing 50 pmol of each primer, 25µl of 2x Master mix (Accu power® PCR PreMix, BioNeer, Korea), 100 ng of DNA template and 22 µl of nuclease-free water.

The amplification thermo cycle parameters condition were, 1min at $94C^0$, followed by 40 cycles of 45s at 94 C^0, 45s at 52 C^0 and 45 s at $72C^0$ and final extention of 7min at $72C^0$ [10].

Samples were loaded carefully into the individual wells of gel agarose, (Promega, USA) stained with ethidium bromide and then electrical power was turned and identified by comparison with reference markers[10].

RESULTS AND DISSCUSION

The total samples (n= 145) were collected from patients with differented cases. The results showed that out of (145) samples 22(15%) of them were positive for *B. Fragilis* as presumptive isolates by using macroscopic and microscopic examination in addition to traditional biochemical tests, fourteen samples 15(10.3%) were positive for *B. Fragilis* and by using PCR methods for detection enterotoxin, maximum positive diarrhea cases were 6(11.7%), Colorectal cancer 5(11%), abdominal surgical abscesses 3(9.7%), Vaginal infection 1(5,6%) cases respectivelly .The disribution of *B.fragilis* infection according to the cases, the result shown a highly significant increase infection at diarrhea infection in children less than 5 years of age. (Table: 1).

Culture examination was done on Columbia blood agar under anaerobic conditions, colonies appeared smooth, white to gray, non hemolytic and (1-3mm) in

Table 3: Distribution of Sample Study According to Antibiotic Sensitive .

Antibiotic (µg/disc)	Sensitive No. (%)	Intermediate No. (%)	Resistance No.(%)
Metronidazole, MTZ(5)	22(100%)	00	00
Chloramphenicol , C (10)	22(100%)	00	00
Rifampin, Rif (5)	18(81.8)%	00	4(18.1%)
Ampicillin, AM(25)	00	00	22(100%)
Vancomycin, VA (30)	2(9. 09%)	00	20(90.9%)
Erythromycin, E (15)	3(13.6%)	00	19(86.3%)

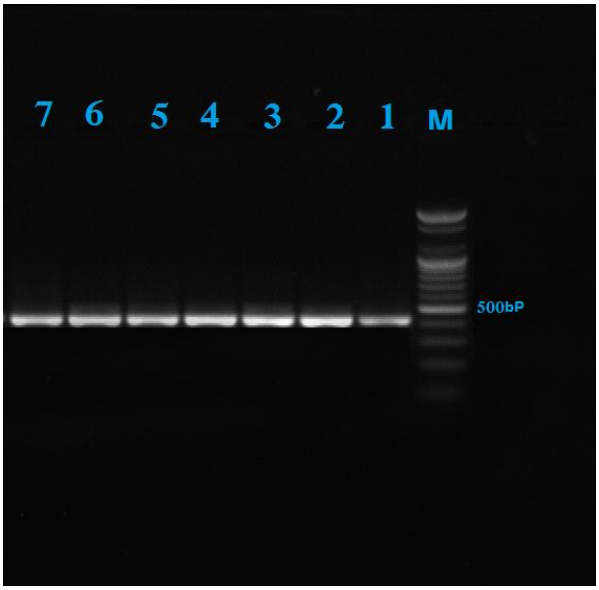

Figure 1: Agarose gel electrophoresis of PCR amplification products of *B. Fragilis* ,expected products of amplification were 400bp.

diameter, all isolates were gave gram positive, rod shaped cells, identification by using Biochemical test to further confirmation with the bacteria, API 20A kits (bio Mérieux)was used . After 48 hrs. of incubation all the isolates were confirmed, Table[2] .

Enterotoxigenic *B. Fragilis* (ETBF) induced diarrhea in children has been reported by different researchers in various parts of the world, and suggest that detection of ETBF may be a potential marker of early colorectal carcinogenesis, the similar result were recorded by [1,8,11,12,13,19], and mention that enterotoxigenic *B. fragilis* could be a cause for vaginitis[20].

It also tests the susceptibility of the bacterial isolates aginst (6) types of the antibiotic . The results appear that the *B. Fragilis* bacteria are extremely resistant to numerous antibiotics, so it appears a ratio of resistance to antibiotics (Ampicillin, Vancomycin, Erythromycin) and these results coincides with pervious study[14,15, 16], whereas show a highly sensitive against the antibiotics (Metronidazole**,** Chloramphenicol**,** Rifampin)[17,18,8].

CONCLUSION

The results showed that *B. fragilis* was the most prevalent species in diarrheal stools samples and important anaerobic pathogens that frequently cause various infections, and due to the decrease in sensitivity to antibiotics, selection of antibiotic has been difficult. The chloramphenicol, and metronidazole remain active against *B. Fragilis* isolates.

REFERENCES

1. Akhi, M.T., Shirinzadeh, M., Ghotaslou, R., Sorous, M.H., Pirzadeh, T., Naghavi, B. M. (2013). Determination of Antibiotic Sensitivity of *Bacteroid fragilis* Isolated from Patients and Healthy Individuals in Imam Reza Center of Medical Teaching and Treatment-Tabriz. *Jundishapur J Microbiol.*; 6(9): 960–3.
2. 2.Rodrigues, C., Siciliano,R.F., Zeigler,R., Strabelli, T.M.(2012). *Bacteroides fragilis* endocarditis: a case report and review of literature. *Braz J Infect Dis.* ;16(1):100–4.
3. Merino V.R., Nakano V., Liu C., Song Y., Finegold S.M., Avila-Campos M.J.(2011). Quantitative detection of enterotoxigenic *Bacteroides fragilis* subtypes isolated from children with and without diarrhea. *J Clin Microbiol.*;49(1):416–8.
4. Akhi M. T., Seifi S. J., Asgharzadeh M., Rezaee M. A., Oskuei S.A., Pirzadeh T., Memar M. Y., Alizadeh N., and Sofla H. S. Y.(2016). Role of Enterotoxigenic *Bacteroides fragilis* in Children Less Than 5 Years of Age With Diarrhea in Tabriz, Iran. *Jundishapur J Microbiol.*; 9(6): e32163.
5. 5- Boleij A., Hechenbleikner E. M., Goodwin A. C., Badani R., Stein E. M., Lazarev M. G., Ellis B., Carroll K. C., Albesiano E., Wick E. C., Platz E. A., Pardoll D. M.,and Sears C. L.(2015). The *Bacteroides fragilis* Toxin Gene Is Prevalent in the Colon Mucosa of Colorectal Cancer Patients. *Clinical Infectious Diseases*;60(2):208–15.
6. Fathi P. and Shaoguang Wu.(2016) . Isolation, Detection, and Characterization of Enterotoxigenic *Bacteroides fragilis* in Clinical Samples. *The Open Microbiology Journal*, 10, 57-63.
7. Fernández-Canigia L., Litterio M., Legaria M.C., Castello L., Predari S.C., Di Martino A. (2012). First national survey of antibiotic susceptibility of the Bacteroides fragilis group: emerging resistance to carbapenems in Argentina. Antimicrob Agents Chemother,56:1309-14.
8. Yehya M., Hamze M. , Mallat H., Dabbousi F.(2013). Prevalence and antibiotic susceptibility of *Bacteroidesfragilis* group isolated from stool samples in North Lebanon. *Brazilian Journal of Microbiology*, 44(3): 807-812.
9. British Society for Antimicrobial Chemotherapy (BSAC) (2011). Methods for antimicrobial

susceptibility testing, Version 10.2, *BSAC*, Birmingham, United Kingdom.

10. Toprak N. U, Yagci A., Gulluoglu B. M., Akin M. L., Demirkalem P., Celenk T. and Soyletir. G. (2006). A possible role of *Bacteroides fragilis* enterotoxin in the aetiology of colorectal cancer. *Clin Microbiol Infect*, 12: 782–786.
11. Jiang Z.D, Dupont H.L., Brown E.L., Nandy R.K., Ramamurthy T., Sinha A.(2010) . Microbial etiology of travelers' diarrhea in Mexico, Guatemala, and India: importance of enterotoxigenic *Bacteroides fragilis* and Arcobacter species. *J Clin Microbiol.*,48(4):1417–9.
12. Merino V.R., Nakano V., Liu C., Song Y., Finegold S.M., Avila-Campos M.J.(2011). Quantitative detection of enterotoxigenic *Bacteroides fragilis* subtypes isolated from children with and without diarrhea. *J Clin Microbiol.*;49(1):416–8.
13. Ramamarthy, D.; Pazhani, G.; Sarker, A.; Nandy, K. R.; Rajendran, K.; Sur, D. and Ramammurthy, T. (2013). Case Control study on the Role of Entertoxigenic *Bacterooides fragilis* as acase of diarrhea among children in Kalkata, India. DOI: 10 . 173/1/Journal.
14. Jamal, W.; AL-Hashem, G. and Rotimi, O. V.(2014). Antimicrobial resistance among anaerobes isolate from clinical specimens in Kuwait hospitals : comparative analysis of year data. Anaerobes. Antibiotic, Resistance, Kuwait.
15. Stiefel, U.; Nerandzic, M. M.; Pultz, J. M. and Ponskey, J. C. (2014). Gastrointestinal colonization with a cephalosporinase-producing Bacteroides species preserves colonization resistance agents vancomycin–risistance Enterococcus and *Clostridiam difficile* in cephalosporin treated mice. DOI: 10. 1128/AAC. 02782-14.
16. Galvao, B. P.; Meggersee. R. L. and Abratt, V. R. (2011). Antibiotic resistance and adhesion potential *Bacteroides fragilis* clinical isolates from Cape–Town, South Africa. Anaerobe. 17(4): 142-6.
17. Wang, D. F.; Liao, H. C.; Lin, T. Y.; Sheng, H. W. and Hsueh, R. P. (2014). Trends in the susceptibility of commonly encountered clinicly significant anaerobes to 16 antimicrobial agents, including fidaxomicin and rifaximin, Northern-Tawin. J. Clin. Microbial infect Dis. 33:2041-2052.
18. Akhi, M. T.; Chotaslou, R.; Shirinzadeh, M.; Pirzadeh, T.; Behazad, N. M. (2013). Comparision of E-test and disk diffusion test for antibiotic resistance testing of enterotoxigenic and non-enterotoxigenic *Bacteroides fragilis* isolated from stool. Jundishapur Journal of Microbiology. 6(5): e9800.
19. Purcell RV, Pearson J, Aitchison A, Dixon L, Frizelle FA, Keenan JI (2017) . Colonization with enterotoxigenic *Bacteroides fragilis* is associated with early-stage colorectal neoplasia. PLoS ONE 12(2): e0171602.
20. Polanco N, Manzi L, Carmona O.(2012). Possible role of enterotoxigenic Bacteroides fragilis in the etiology of infectious vaginitis. Invest Clin. Mar;53(1):28-37.